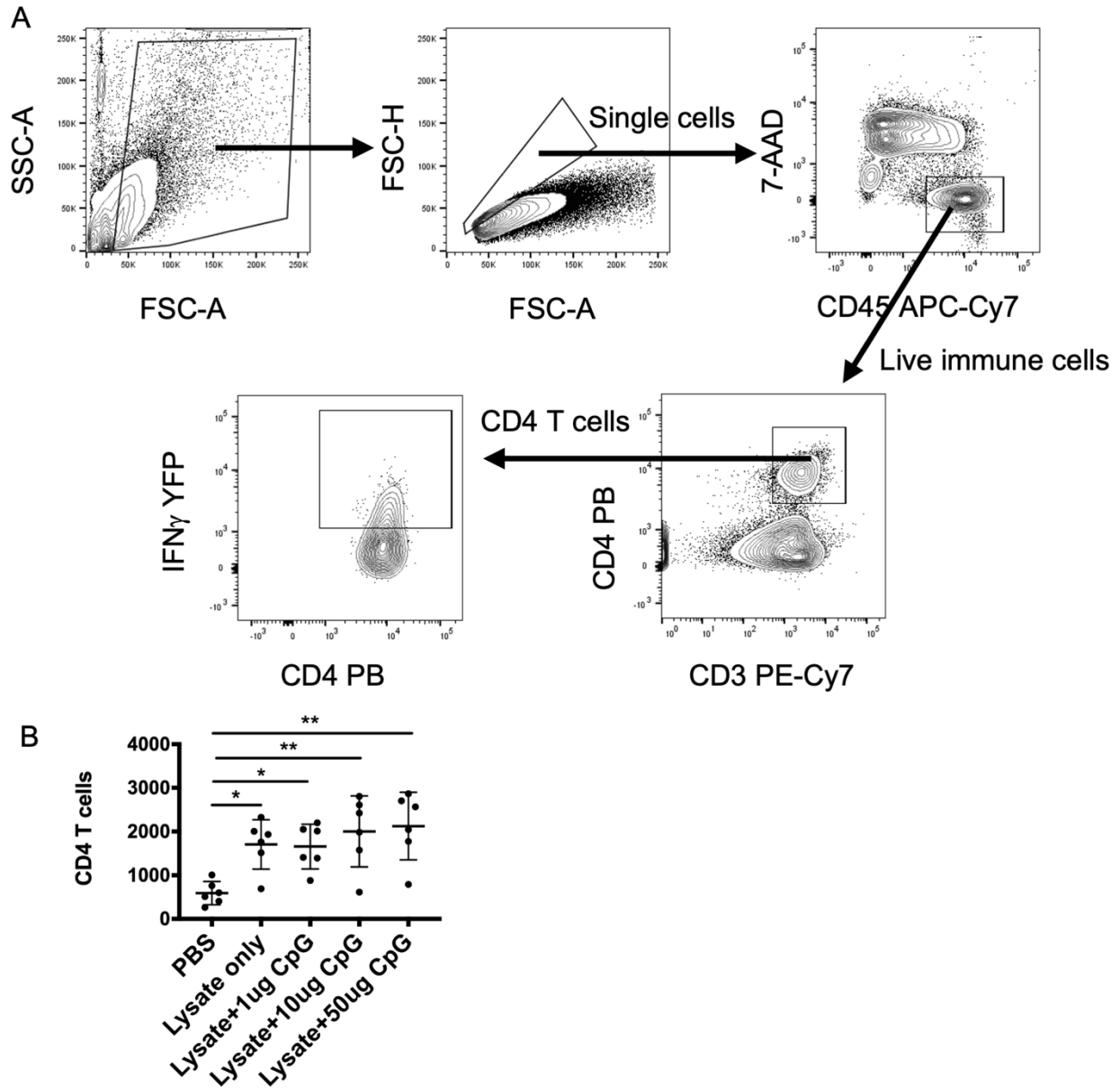


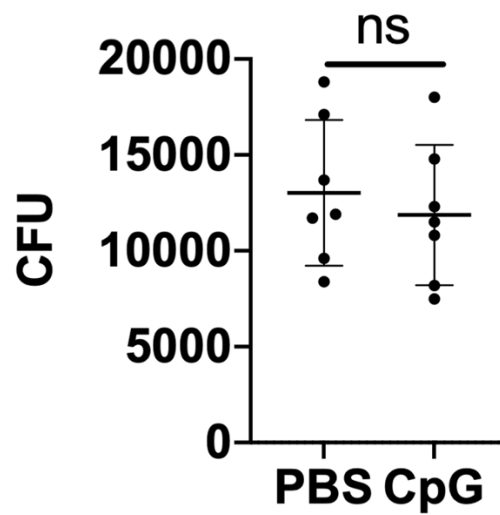
Supplement figure 1. Heat lysis treatment results in complete loss of bacteria viability.  $10^8$  CFU of bacteria in PBS were either placed at room temperature (Ctrl) or treated over  $60^\circ\text{C}$  (Heat Lysis) for 30min, thereafter bacterial viability was assessed by colony counts on Difco MacConkey agar plate. After overnight incubation in  $37^\circ\text{C}$ , colony forming unit (CFU) was determined. Each data point represents one sample, data are shown as mean  $\pm$  SD. Data were analyzed by an unpaired t test. \*\*\* $p < 0.001$ .



Supplement figure 2. Flow cytometry analysis of CD4 T cells in immunized bladder.

A. Gating strategy was based on a previous study (7). Briefly, single cells were first gated based on FSC and SSC. Then, live immune cells were gated as 7-AAD<sup>-</sup>CD45<sup>+</sup>. Finally, CD3<sup>+</sup>CD4<sup>+</sup>IFN $\gamma$ <sup>+</sup> cells were analyzed.

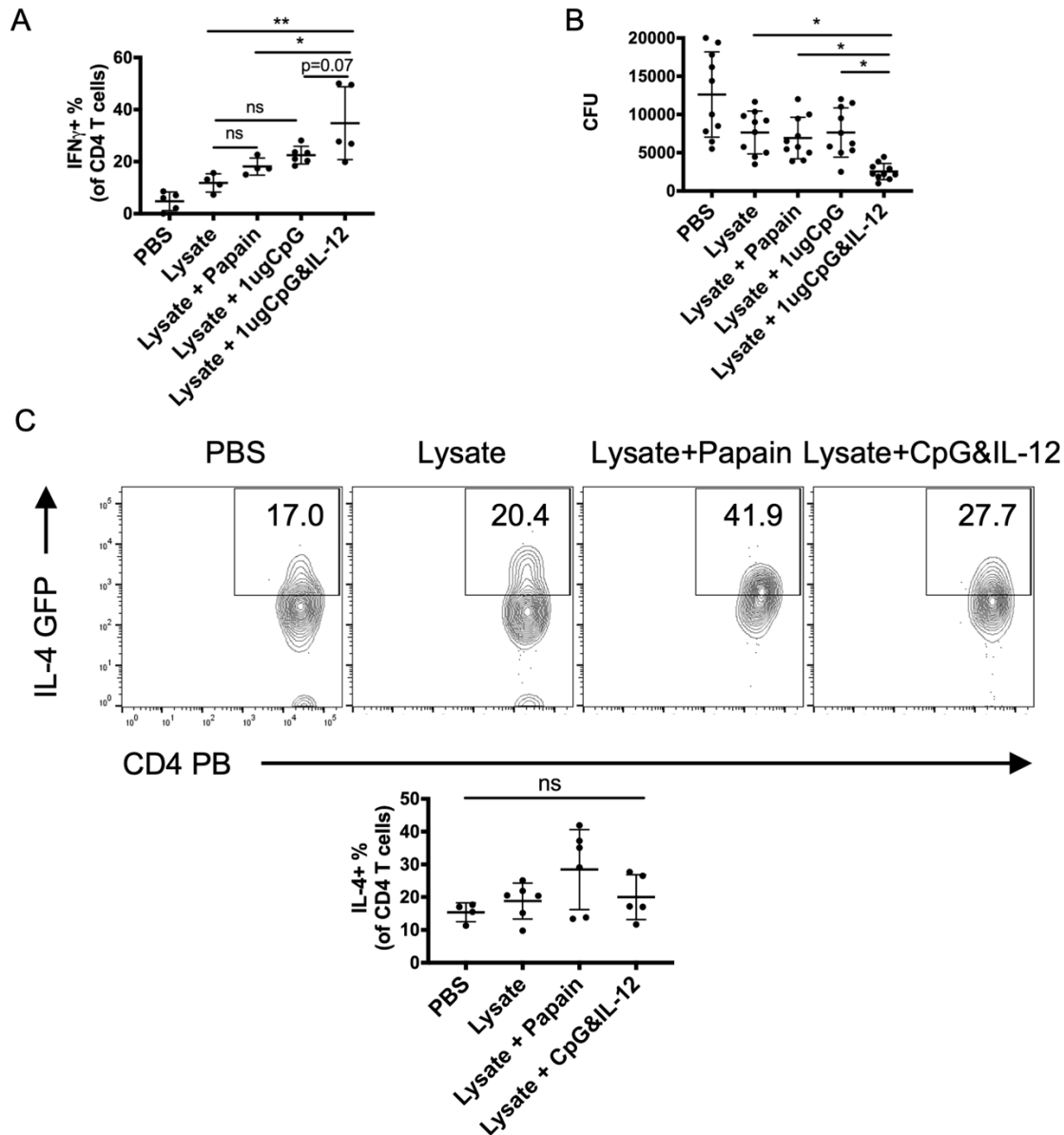
B. The same immunizations were performed as figure 1. The number of total CD4 T cells in each bladder of each immunization group was shown. Each data point represents one mouse, data are shown as mean  $\pm$  SD. Data were analyzed by an ordinary one-way ANOVA with the Tukey's multiple comparison post-hoc test. \* $p < 0.05$ , \*\* $p < 0.01$ .



Supplement figure 3. Intravesical vaccination with CpG alone does not improve bacterial clearance.

Naïve WT mice were intravesically vaccinated with PBS or 10ug CpG three times, then intravesically challenged with UPEC J96. Bladders were collected to determine bacterial load on day 3 post challenge.

Each data point represents one mouse, data are shown as mean  $\pm$  SD. Data were analyzed by an unpaired t test. ns=not significant.



Supplement figure 4. Combination of CpG and IL-12 with J96 lysate induces a strong Th1 response but does not inhibit the Th2 response.

A. Naïve Great mice were intravesically vaccinated with UPEC J96 lysates combined with 10ug Papain, 1ug CpG, or 1ug CpG & 10ug IL-12 (to make sure the total amount of adjuvant used is round 10ug in this group) three times. PBS or UPEC J96 lysates alone treated mice served as controls. Then these mice were challenged with UPEC J96. Bladders were collected for flow cytometry analysis on day 3 post challenge. Please note that the data of UPEC lysates+1ugCpG are the same from Figure 1A.

B. Naïve WT mice were intravesically vaccinated with UPEC J96 lysates with 10ug Papain, 1ug CpG, or 1ug CpG & 10ug IL-12 three times. PBS or J96 lysate alone treated mice were controls. Then all mice were challenged with UPEC J96 and bacterial load determined on day 3.

C. Naïve 4get mice were intravesically vaccinated with UPECJ96 lysate with Papain or CpG & IL-12 three times. PBS or J96 lysate alone treated mice served as controls. There after these mice were challenged with UPECJ96. Bladders were collected for flow cytometry analysis on day 3 post challenge.

Each data point represents one mouse, data are shown as mean  $\pm$  SD. Data were analyzed by an ordinary one-way ANOVA with the Tukey's multiple comparison post-hoc test. \* $p < 0.05$ , \*\* $p < 0.01$ , ns=not significant.